A MICROMANIPULATOR FOR THE ISOLATION OF BACTERIA AND THE DISSECTION OF CELLS

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I have recently described (Chambers, 1922, b) an apparatus for the manipulation of micro needles and micro pipettes under the highest magnifications of the microscope. This apparatus is an improvement on Barber's Pipette Holder (Barber, 1914) because of its simpler construction and the greater accuracy with which one can control its movements. An additional advantage consists in the existence of certain devices for bringing the pipette or needle, quickly into position before starting actual operation.

The working principle of the apparatus (which is being patented) is illustrated in figure 1. It consists in the use of bars of rigid metal connected at their ends to form a Z like figure by resilient metal acting as spring hinges. The bars are forced apart by screws and return when the screws are reversed. By these means arc movements are imparted to the tip of a pipette which is attached to one of the bars. As the radius of each arc is about two and a half inches, the fine movements imparted to the tip of the pipette are practically in straight lines because the excursion never exceeds one millimeter.

The instrument can be used by itself for one needle or pipette, or with a companion apparatus when two needles, or a needle and a pipette are to be used simultaneously. When a pair is used, one is a left handed and the other a right handed apparatus, both being clamped to the front of the microscope stage. For the isolation of bacteria, one instrument is sufficient. It may be clamped on the left side of the microscope stage, figure 2, so that the pipette projects into the moist chamber from the

left. The tip of the needle or pipette is bent up so as to project from below into a drop suspended from the coverslip which roofs the chamber. The cells to be operated upon lie in the hanging drop. When a cell is to be dissected or injected it tends to retain its position on account of the shallowness of the drop and the inertia of the cell. However, it is more satisfactory to use two instruments, one with a needle for holding the cell or tissue, and the other with a needle or pipette for the actual operation.

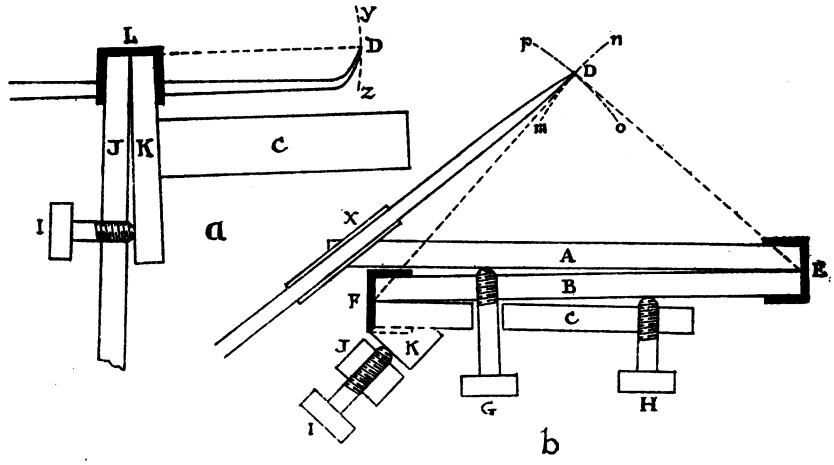

FIG. 1. DIAGRAM SHOWING WORKING PRINCIPLE OF MICROMANIPULATOR

(a) Side view. Screw *I* in stationary pillar *J* pushes against *K*, and causes needle tip *D* to move through vertical arc *y-z*.

(b) Surface view. Screws *G* and *H* move the needle tip through horizontal arcs *m-n* and *o-p*.

For dissecting purposes, the glass needles may be curved or straight and with obtusely or gently tapering tips. They can be made fine enough to puncture red blood corpuscles and to tear up leucocytes.

For injecting and for withdrawing materials from a living cell, the micro pipettes are made with apertures varying from two to less than half a micron in diameter. I have recently described (Chambers, 1922, a) an effective and easily made apparatus for exerting the necessary pressure to drive materials through

such small pipettes, and at the same time to control, with considerable accuracy, the amount to be injected or withdrawn.

For isolating bacteria, much coarser pipettes are used, which can be blown into by the mouth through a length of rubber tubing, figure 2. At my suggestion, Dr. Kahn has kindly pub-

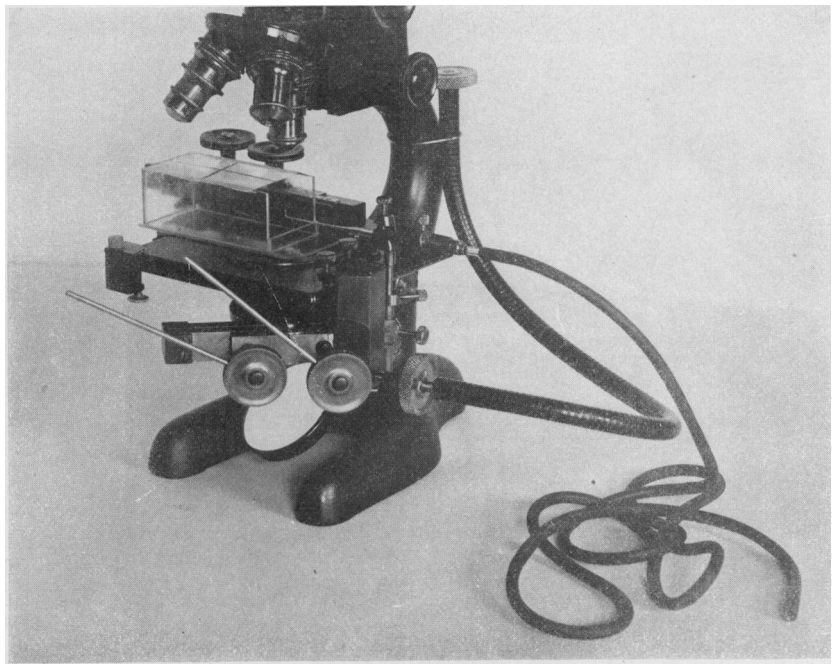

FIG. 2. MICROMANIPULATOR MOUNTED ON LEFT SIDE OF MICROSCOPE FOR ISOLATING BACTERIA

Note Barber's moist chamber with the coverslip marked with cross lines to aid in locating areas. The chamber shown here is higher than necessary. The screw producing the vertical movement is connected with a flexible shaft, which allows its control to be brought into close proximity with the fine adjustment of the microscope.

lished an account (Kahn, 1922) of the procedure, together with a discussion of the application of the micromanipulator to the isolation of bacteria. In brief, the procedure is as follows: A sterile, hollow glass needle is first made. The bent up tip is then inserted into a test tube of a liquid culture of bacteria,

and converted into a pipette by breaking the tip against the wall of the test tube. A small amount of the culture is sucked up, and the filled pipette placed in the micromanipulator attached to the microscope. The tip of the pipette is then brought into the microscopic field and brought close to the coverslip of the

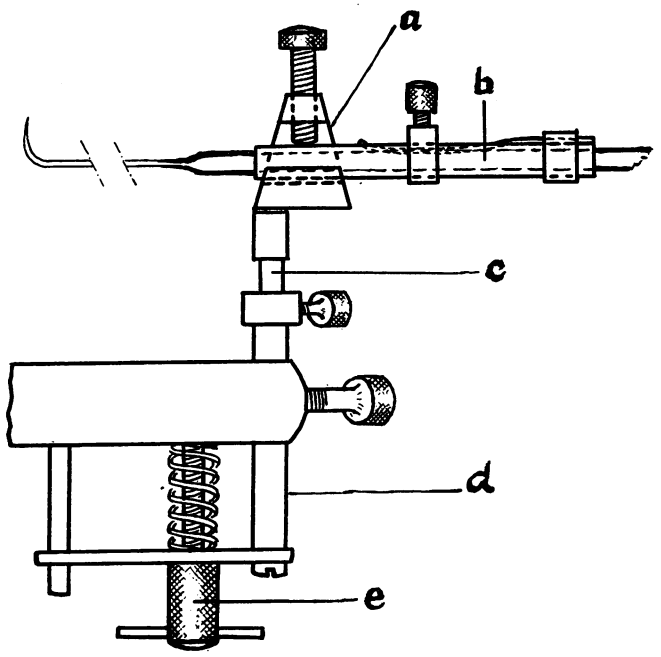

FIG. 3. DETAIL SHOWING DEVICES FOR PRELIMINARY ADJUSTMENTS OF THE PIPETTE

Carrier, *a*, for clamping brass collar, *b*, in which needle or pipette has been inserted. The needle or pipette slides evenly within the collar for the in and out movement. Telescoping pillar, *c*, for lengthening vertical post of carrier. Post, *d;* rotates and serves to move needle tip laterally, Screw, *e*, raises and lowers post, *d*, to move needle tip vertically.

moist chamber by means of the preliminary adjusting devices shown in detail in figure 3. The tip is now further raised by means of the fine adjustment screw until it reaches the undersurface of the coverslip. By alternately raising and lowering the pipette, and by moving the moist chamber with the mechani-

cal stage, a series of hanging droplets[1] are placed on the coverslip. The pipette is then removed from the instrument and discarded. A search is now made for droplets containing only a single organism. Each such droplet is drawn up into a fresh sterile pipette, which is then removed from the instrument and inserted into a tube containing a suitable sterile medium. The contents of the pipette are now expelled by blowing. In this way, one can quickly obtain cultures known to have originated from a single organism.

The micromanipulation technic is not very difficult. The making of the glass needles and pipettes, and the working of the instrument can be quickly mastered.

For the bacteriologist, the isolation method as introduced by Barber, has long proved most successful. With the apparatus described here, it should soon be more widely used.

For the cytologist and cell physiologist, the problem is to find the proper material with which to work. Through microoperations on certain tissue cells and on such material as Protozoa and marine ova, considerable light has already been thrown upon the nature of living protoplasm.

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[1] Barber uses coverslips smeared with petrolatum to aid in the maintenance of the droplets. The excess, having been washed off with soap and water, the slips are dried with a cloth, and then heated and wiped a second time while still warm. They are sterilized by flaming.